Amendments to the Specification:

Please replace paragraphs 18, 19, 20, 22, 40, 77, 88, 183, and 204 with the following amended paragraphs:

[0018] One embodiment of the invention provides nucleic acids encoding Diphtheria toxin fusion proteins comprising (1) residues 1-388 of Diphtheria toxin, wherein the native furin cleavage site has been substituted for a cleavage site for matrix metalloproteinase or a plasminogen activator and (2) a heterologous polypeptide that binds to a protein expressed on the surface of the cell. In some embodiments, the matrix metalloproteinase is MMP-2 (gelatinase A), MMP-9 (gelatinase B) or membrane-type 1 MMP (MT1-MMP). In some embodiments, the tissue plasminogen activator is tPA (tissue-type plasminogen activator) or u-PA (urokinase-type plasminogen activator) . In some embodiments, the heterologous polypeptide is a cytokine (*e.g.*, IL-2, GM-CSF, IL-4, IL-5, IL-6, IL-10, or IL-12) or a growth factor (*e.g.*, epidermal growth factor (EGF), transforming growth factor (TGF), or fibroblast growth factor (FGF)). In some embodiments, the nucleic acid encodes a Diphtheria toxin fusion protein comprising (1) residues 1-388 of Diphtheria toxin, wherein the native furin cleavage site has been substituted for a cleavage site for uorkinase plasminogen activator and (2) GM-CSF. In some embodiments, the nucleic acids comprise the nucleotide sequences set forth in any one of SEQ ID NOS: 2-13. In addition, the invention provides vectors comprising the nucleic acids described above, and host cells comprising such vectors. The invention also provides proteins and polypeptides encoded by the nucleic acids described above. The invention further provides pharmaceutical compositions comprising polypeptides encoded by the nucleic acids described above and a pharmaceutically acceptable carrier.

[0019] Another embodiment of the invention provides a method of treating cancer by administering to a subject a Diphtheria toxin fusion protein comprising (1) residues 1-388 of Diphtheria toxin, wherein the native furin cleavage site has been substituted for a cleavage site for matrix metalloproteinase or a plasminogen activator and (2) a heterologous polypeptide that binds to a protein expressed on the surface of the cell. In some embodiments, the matrix metalloproteinase is MMP-2, MMP-9, or MMP1-MMP. In some embodiments, the tissue

plasminogen activator is tPA or uPA. In some embodiments, the matrix metalloproteinase-recognized cleavage site is GPLGMLSQ (SEQ ID NO: 19) or GPLGLWAQ (SEQ ID NO: 20). In some embodiments, the heterologous polypeptide is a cytokine (*e.g.*, IL-2, GM-CSF, IL-4, IL-5, IL-6, IL-10, or IL-12) or a growth factor (*e.g.*, EGF, hGF, or FGF). In another embodiment, the plasminogen activator-recognized cleavage site is GSGRSA (SEQ ID NO: 21), GSGKSA (SEQ ID NO: 22), or QRGRSA (SEQ ID NO: 23). In some embodiments, the Diphtheria toxin fusion protein comprises (1) residues 1-388 of Diphtheria toxin, wherein the native furin cleavage site has been substituted for a cleavage site for uorkinase plasminogen activator and (2) GM-CSF. In some embodiments, the DT fusion protein is encoded by the nucleotide sequence set forth in any one of SEQ ID NOS: 2-13. In some embodiments, the cancer is acute myelogenous leukemia, monocytic leukemia, lung cancer, breast cancer, bladder cancer, thyroid cancer, liver cancer, lung cancer, pleural cancer, pancreatic cancer, ovarian cancer, cervical cancer, colon cancer, fibrosarcoma, neuroblastoma, glioma, or melanoma.

[0020] A further embodiment of the invention provides a method of targeting a compound to a cell overexpressing a cytokine receptor or a growth factor receptor by administering to the cell a Diphtheria toxin fusion protein comprising Diphtheria toxin fusion protein comprising (1) residues 1-388 of Diphtheria toxin, wherein the native furin cleavage site has been substituted for a cleavage site for matrix metalloproteinase or a plasminogen activator and (2) a heterologous polypeptide that binds to a protein expressed on the surface of the cell. In some embodiments, the matrix metalloproteinase is MMP-2, MMP-9, or MMP1-MMP. In some embodiments, the tissue plasminogen activator is tPA or uPA. In some embodiments, the matrix metalloproteinase-recognized cleavage site is GPLGMLSQ (SEQ ID NO: 19) or GPLGLWAQ (SEQ ID NO: 20). In some embodiments, the heterologous polypeptide is a cytokine (*e.g.*, IL-2, GM-CSF, IL-4, IL-5, IL-6, IL-10, or IL-12) or a growth factor (*e.g.*, EGF, hGF, or FGF). In another embodiment, the plasminogen activator-recognized cleavage site is GSGRSA (SEQ ID NO: 21), GSGKSA (SEQ ID NO: 22), or QRGRSA (SEQ ID NO: 23). In some embodiments, the cell also overexpresses a matrix metalloproteinase or a plasminogen activator. In some embodiments, the Diphtheria toxin fusion protein comprises (1) residues 1-388 of Diphtheria

toxin, wherein the native furin cleavage site has been substituted for a cleavage site for uorkinase plasminogen activator and (2) GM-CSF.

[0022] Fig. 2 depicts a schematic illustration of mutant DT fusion proteins that can be specifically processed by MMPs. Human GM-CSF was recombinantly fused to the C-terminus of modified DT388. The table represents the sequence modified in the furin sensitive surface loop (SEQ ID NO: 24) of DTGM that generate cleavage sites recognized by furin (SEQ ID NO: 28), uPA (SEQ ID NOS: 25 and 26), or MMP (SEQ ID NO: 27) as indicated. To generate DTGM-U2, the native furin cleavage site was replaced by GSGRSA (SEQ ID NO: 21), a urokinase plasminogen cleavage site. To generate DTGM-U3, the native furin cleavage site was replaced by GSGKSA (SEQ ID NO: 22), a urokinase plasminogen cleavage site. To generate DTGM-L1, the native furin cleavage site was replaced by GPLGMLSQ (SEQ ID NO: 19), a matrix metalloproteinase cleavage site.

[0040] The terms "Diphtheria toxin" and "DT" refer to a 535 amino acid polypeptide secreted by *Corynebacterium diphtheriae* or a subsequence thereof. DT comprises three domains: (1) an N terminal catalytic domain (aa 1-186); (2) a translocation domain (aa 187-388); and (3) a cell binding domain (aa 389-535) (*see, e.g.*, Frankel *et al.*, *Protein Peptide Lett.* 9(10:1-14 (2002) and Genbank Accesion No. A04646). The catalytic domain and translocation domain are connected by a furin-sensitive disulfide loop. DT binds to a heparin-binding epidermal growth factor-like growth factor expressed on a cell surface via the cell binding domain and associates with CD9 and heparin sulfate proteoglycan. The DT complex is then internalized into the cell (*i.e.*, into endosomes). Once in the endosomal compartment, DT undergoes furin cleavage which releases the catalytic domain which is then translocated through the endosomal membrane and into the cytosol. Once in the cytosol, the catalytic domain ASP-ribosylates elongation factor 2 (EF2), thus blocking protein synthesis and killing the cell.

[0077] Examples of protocols sufficient to direct persons of skill through in vitro amplification methods, including the polymerase chain reaction (PCR) the ligase chain reaction (LCR), Qβ-replicase amplification and other RNA polymerase mediated techniques are found in Berger, Sambrook, and Ausubel, as well as Mullis *et al.* (1987) U.S. Patent No. 4,683,202; PCR

PROTOCOLS A GUIDE TO METHODS AND APPLICATIONS (Innis *et al.* eds) Academic Press Inc. San Diego, CA (1990) (Innis); Arnheim & Levinson (October 1, 1990) C&EN 36-47; The Journal Of NIH Research (1991) 3: 81-94; (Kwoh *et al.* (1989) *PNAS USA* 86: 1173; Guatelli *et al.* (1990) *PNAS USA* 87: 1874; Lomell *et al.* (1989) *J. Clin. Chem.* 35: 1826; Landegren *et al.* (1988) *Science* 241: 1077-1080; Van Brunt (1990) *Biotechnology* 8: 291-294; Wu and Wallace (1989) *Gene* 4: 560; and Barringer *et al.* (1990) *Gene* 89: 117. Improved methods of cloning in vitro amplified nucleic acids are described in Wallace *et al.*, U.S. Pat. No. 5,426,039.

[0088] DT fusion proteins can be produced from nucleic acid constructs encoding amino acid residues 1-388 of DT, in which the naturally occurring furin cleavage site has been replaced by an MMP or a plasminogen activator cleavage site, and a hterologous polypeptide (*e.g.*, GM-CSF, IL-2, or EGF). Those of skill in the art will recognize a wide variety of ways to introduce mutations into a nucleic acid encoding DT toxin or to construct a mutant DT toxin-encoding nucleic acid. Such methods are well known in the art (*see* Sambrook *et al.*, *Molecular Cloning, A Laboratory Manual* (2nd ed. 1989); Kriegler, *Gene Transfer and Expression: A Laboratory Manual* (1990); and *Current Protocols in Molecular Biology* (Ausubel *et al.*, eds., 1994)). In some embodiments, nucleic acids of the invention are generated using PCR. For example, using mutagenic PCR DT fusion protein encoding nucleic acids can be generated by substituting the nucleic acid subsequence that encodes the furin site with a nucleic acid subsequence that encodes a matrix metalloproteinase (MMP) site (*e.g.*, GPLGMLSQ (SEQ ID NO: 19) and GPLGLWAQ (SEQ ID NO: 20)). Similarly, a mutagenic PCR method can be used to construct the DT fusion proteins in which the furin site is replaced by a plasminogen activator cleavage site (*e.g.*, the uPA and tPA physiological substrate sequence PCPGRVVGG (SEQ ID NO: 29), the uPA favorite sequence GSGRSA (SEQ ID NO: 21), the uPA favorite sequence GSGKSA (SEQ ID NO: 22), or the tPA favorite sequence QRGRSA (SEQ ID NO: 23)).

[0183] Human GM-CSF was recombinantly fused to the C-terminus of modified DT388. The table represents the sequence modified in the furin sensitive surface loop of DTGM that generate cleavage sites recognized by furin, uPA, or MMP as indicated. To generate DTGM-U2, the native furin cleavage site was replaced by GSGRSA, a urokinase plasminogen cleavage site.

To generate DTGM-U3, the native furin cleavage site was replaced by GSGKSA (SEQ ID NO: 22), a urokinase plasminogen cleavage site . To generate DTGM-L1, the native furin cleavage site was replaced by GPLGMLSQ (SEQ ID NO: 19), a matrix metalloproteinase cleavage site.

[0204] The first generation of DT fusion toxins (**i.e.*, DT_{388}GMCSF, DT_{388}IL3 and DTAT) for AML have only a single targeting moiety – the ligand - and were potentially toxic to normal tissues bearing their respective receptors, GMCSFR, IL3R or uPAR (*see, e.g.*, Frankel *et al.*, *Protein Expr. Purif.* 16(1):190-201 (1999); Ramage *et al.*, *Leukemia Research* 27(1): 79-84 (2003); and Urieto *et al.*, *Protein Expr Purif.* 33(1): 123-33 (2004)). Clinical testing of the AML fusion toxin DTGM confirmed clinical efficacy but with associated damage to GMCSFR containing normal cells. This led to significant liver injury. We replaced DT $_{163}$RVRRSV$_{170}$ (SEQ ID NO: 30) with the uPA cleavage sequence $_{163}$GSGRSA$_{170}$ (SEQ ID NO: 21) in DTGM, to generate a new AML fusion toxin DTGM-U2 that would retain potency but have enhanced AML specificity. The new dual specificity fusion toxin DTGM-U2 was produced in the same yields and purity as DTGM. The protein remained stable and biologically active after storage at -80° C for over one month. Further, DTGM-U2 remained fairly stable after incubation with serum at 37°C for 48 hours (IC_{50} = 12.8 pM as compared to an IC_{50} = 6.3 pM for freshly thawed DTGM-U2 in a tritiated thymidine incorporation inhibition assay on HL60 cells).

At page 63, line 30, replace "DT-GML1" with --DTGM-L1--

At page 64,

line 13, replace "DT-GML2" with --DTGM-L2--;

line 37, replace "DT-GMU2" with --DTGM-U2--.

At page 65,

line 2, replace "DT-GMU3" with --DTGM-U3--; and

line 26, replace "DT-EGFL1" with --DGEGF-L1.

At page 67,

Line 6, replace "DT-EGFL2" with --DTEGF-L2--; and

Line 27, replace "DT-EGFU2" with --DTEGF-U2--.

At page 68,

Line 7, replace "DT-EGFU3" with --DTEGF-U3--; and

Line 28, replace "DT-IL2L1" with --DTIL2-L1--.

At page 69,

Line 12, replace "DT-IL2L2" with --DTIL2-L2--; and

Line 37, replace "DT-IL2U2" with DTIL2-U2.

At page 70, line 20, replace "DT-IL2U3" with --DTIL2-U3--.